Interanal seam loss in Asian turtles of the *Cuora flavomarginata* complex (Testudines, Geoemydidae)

CARL H. ERNST[1*] & JEFFREY E. LOVICH[2]

[1]Division of Amphibians and Reptiles, mrc 162, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013-7012, USA

[2]U.S. Geological Survey, Southwest Biological Science Center, 2255 North Gemini Drive, Flagstaff, Arizona 86001, USA

[*]Corresponding author email: chernst@frontiernet.net

ABSTRACT - The taxonomy of Asian box turtles of the genus *Cuora* is complicated by the description of numerous valid and invalid taxa over the last several decades. However, some characteristics used to differentiate species are questionable. Members of the *C. flavomarginata* complex are defined by some, but not all, taxonomists as having reduced interanal seam lengths relative to other species. We examined the ratio of interanal scute seam length divided by midline anal scute length in *C. flavomarginata* and *C. evelynae*. Hatchlings show a seam that divides 100% of the anal scute along the midline. As individuals increase in carapace length, there is a tendency for the percentage to decrease, especially in females, although there is considerable overlap. We suggest that the decrease in interanal seam length is due to abrasion of the plastron on the substrate as turtles grow larger and older. Differences in habitat substrates across the range of the species may contribute to the wide variation we observed.

INTRODUCTION

Turtles of the Asian genus *Cuora* have a complicated taxonomic history, in large measure due to the description of hybrid forms as distinct taxa (Parham et al., 2000; Parham et al., 2001; Shi et al., 2005; Stuart & Parham, 2007). Twelve species are currently recognized with 17 terminal taxa including all currently recognized species and subspecies (van Dijk et al., 2014). Ten of those terminal taxa were described since 1980, a reflection of the increasing resolution possible through application of relatively new molecular techniques (Honda et al., 2002; Tiedemann et al., 2014). Still, the distinctiveness and natural distribution of some taxa remain unresolved (e.g., Ernst et al., 2011). For example, the yellow-margined box turtle (*C. flavomarginata*) has been a subject of taxonomic disagreement. The *C. flavomarginata* complex, as defined by Ernst et al. (2008) to include only the species *C. flavomarginata* (Gray, 1863) and *Cuora evelynae* Ernst & Lovich, 1990, has not met with universal acceptance. Recent authors recognize the consistent differences between the taxa and the vicariant nature of their allopatry, but suggest that the latter is a subspecies of the former (Honda et al., 2002; Ota et al., 2009).

Turtles of the *C. flavomarginata* complex have been recognized by some taxonomists as having reduced interanal seam lengths (relative to midline anal scute length) in many individuals, but this condition has had variable use as a taxonomic character. For example, it has been mentioned in descriptions or shown in illustrations of *C. flavomarginata* by Boulenger (1889), Stejneger (1907), Mao (1971), Bonin et al. (2006), and Ota et al.(2009); and *C. evelynae* by Ernst & Lovich (1990). In contrast, this condition was not included in descriptions by Gray (1863, 1870), Siebenrock (1909), Hsü (1930), Pope (1935), Pritchard (1967, 1979), Obst (1986), Ernst & Barbour (1989), or Zhao & Adler (1993). Is the loss of the interanal seam with shell growth a legitimate and unique characteristic of turtles in this complex? Or is it, as has been suggested by some authors, that the ratio of interanal seam length divided by the midline length of anal scutes (hereafter interanal seam percentage or IANSP), decreases as body size increases because of environmental abrasion over the life of an individual? Neither hypothesis has been satisfactorily studied. In this paper we examine variation in IANSP in the *C. flavomarginata* complex. For consistency with our earlier publications, we refer to members of the complex as species, with full recognition that other authors consider them to be subspecies. The distinction between the two approaches has no effect on our analysis: they are sister taxa either way. We hypothesized that larger turtles would have smaller IANSPs and tested the strength of this relationship in our analyses.

METHODS AND MATERIALS

A total of 125 turtles were examined including: 38 *C. evelynae* from the Ryukyu Islands; and 50 Taiwan and 37 southern China *C. flavomarginata* (see Ernst et al., 2008, for straight-line measurements taken on each specimen). To calculate IANSP we divided seam length between the paired anal scutes on the plastron by midline anal scute length. For comparisons of IANSP, adult males, females and juveniles of the two species were analysed separately due to the potential effect of sexual size dimorphism (Gibbons & Lovich, 1990). Sex was determined by examination of secondary sexual characters. Turtles that did not clearly exhibit these characters were considered to be juveniles. Even though the two *Cuora* taxa are closely related (Ernst & Lovich, 1990; Ernst et al., 2008), we tested for differences in IANSP between them before continuing our analyses.

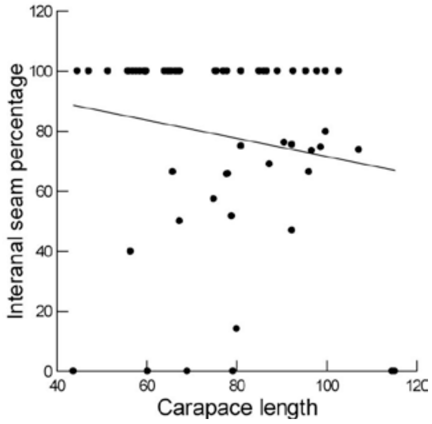

Figure 1. Interanal seam percentage vs. carapace length (mm) of juvenile *C. flavomarginata* and *C. evelynae* combined.

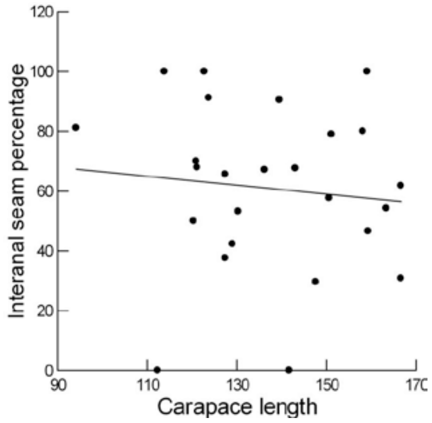

Figure 2. Interanal seam percentage vs. carapace length (mm) of adult male *C. flavomarginata* and *C. evelynae* combined.

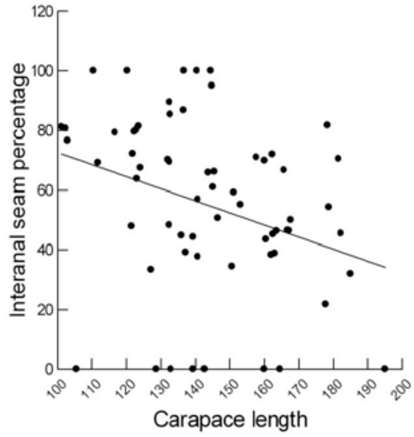

Figure 3. Interanal seam percentage vs. carapace length (mm) of adult female *C. flavomarginata* and *C. evelynae* combined.

Because ratio data do not always satisfy the assumption of normality for parametric tests, we used nonparametric analyses. Spearman correlation coefficients (rs) were calculated to examine the strength of the relationship between carapace length (CL) and IANSP. Since we hypothesized that the correlations between those two variables would be negative (e.g., as CL increased, IANSP decreased) we evaluated test statistics against one-tailed critical values. Mann-Whitney U Tests and Kruskal-Wallis tests were used to compare median values of IANSP between and among sexes and size classes (e.g., males, females and juveniles), evaluating test statistics against two-tailed critical values. Statistical analyses were conducted using STSTAT 12 (version 12.02.00) at an alpha level of 0.05.

RESULTS

The variable IANSP did not differ between *C. flavomarginata* and *C. evelynae* for either females (Mann-Whitney U = 395.0, df = 1, P = 0.78), males (Mann-Whitney U = 76.0, df = 1, P = 0.96), or juveniles (Mann-Whitney U = 195.0, df = 1, P = 0.08), so the taxa were combined for subsequent analyses, but separated according to sex. Values for IANSP varied greatly among females, males, and juveniles (Table 1) but the median and mean values were higher in juveniles than in adults. Median IANSP values were statistically different among juveniles, males, and females (Kruskal-Wallis Test = 8.67, df = 2, P = 0.01). When comparing the median IANSP values for juveniles to the median value for males and females combined, the results were again significant (Mann-Whitney U = 1079.0, df = 1, P = 0.004). However, median IANSP was not significantly different between males and females (Mann-Whitney U = 721.0, df = 1, P = 0.47). Plotting transformed IANSP against CL of juveniles showed a wide scatter with many values of 100% and a few of 0% (Fig. 1). Spearman rank correlation coefficients between CL and IANSP were all negative, as hypothesized. The coefficient was not quite significant for juveniles (rs = -0.26, 0.10 > P > 0.05), or males (rs = -0.18, 0.25 > P > 0.05), the latter as shown in Fig. 2. The coefficient was significant for females (rs = -0.36, P < 0.0025) even though 0-100% values were again recorded across a wide range of CLs (Fig. 3).

	n	Min	Max	Median	Mean	SD
Juveniles	36	0	100	75.3	71.0	33.3
Males	25	0	100	65.6	60.9	27.7
Females	64	0	100	58.1	54.9	29.3

Table 1. Summary statistics for interanal seam percentage in *C. flavomarginata* and *C. evelynae*, combined.

DISCUSSION

The majority of hatchlings of both *C. flavomarginata* and *C. evelynae* that we examined did not have a distinct indented seam between their anal scutes. However, the position where such a seam should occur is usually represented by a medial linear raised area. In this study, we considered this elevated area to represent an interanal seam. Thus, all hatchlings begin life with a 100% IANSP. Examination of hatchling/juveniles with longer CLs, however, revealed an IANSP less than 100% in many, producing a scattering effect (Fig. 1). Such a phenomenon is also revealed when IANSP is plotted against CL of both adult males (Fig. 2) and adult females (Fig. 3). Since adult females are slightly larger than males in mean CL (Gibbons & Lovich, 1990; Ota et al., 2009), the closer values for median and mean IANSP of juveniles and males (Table 1) may be due to some large juveniles being misidentified as males and some small males being misidentified as juveniles.

We suggest that these scattered patterns are indications of wear caused over time when the individual turtle crawls in contact with a hard abrasive substrate in its natural habitat. The turtles we examined are from several localities, likely exposing individual specimens to different substrate textures that cause variation in the rate and extent of shell abrasion. The anal scutes in these *Cuora* taxa are the largest or second largest of the six paired plastron scutes (Ernst et al., 1997) potentially exposing them to a disproportionate amount of wear relative to the other scutes on the bottom of the shell. Turtle shells are well-known to wear smooth with prolonged exposure to abrasive conditions, obfuscating fine annual or semi-annual growth rings on the shell (Germano, 1988; Germano & Bury, 1998) that are often no wider than the contact seam between the anal scutes we examined. Unfortunately, the substrates at the collection sites of the turtles were not recorded, so we have not been able to test this hypothesis. However, if the loss of IANSP with increased CL (i.e., growth) is genetically controlled, the pattern of IANSP should be more predictable than shown in our figures. We conclude that the loss of IANSP in turtles of the *C. flavomarginata* complex is apparently more influenced by environmental microhabitat substrate texture. While the tendency toward a reduced IANSP with size/age does not appear to be genetic and taxonomically useful, many hatchlings/juveniles and some adults retain a 100% IANSP. Adults that retain the full IANSP may live on soft substrates. The presence of the raised linear medial strip between the anal scutes of hatchlings supports this possibility.

We have also recorded loss of IANSP in 92% of the *Cuora galbinifrons* (n=104) we have examined, and only 4% of the *C. amboinensis* (n=671), but not in any other species of *Cuora*. In contrast, we have rarely, if at all, found IANSP loss in the several thousand individuals of emydid species that we have studied during our careers. Consequently, we recommend further study of the possible genetic influence in IANSP in turtles of the genus *Cuora*.

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